Evaluation of nose pressing behaviour in dairy cows in terms of HRV and behaviour

Department für
Nachhaltige Agrarsysteme

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Univeristät für Bodenkultur Wien **-Iñigo Sesma Telleria-**

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SUMMARY

Nose pressing can be described as a behavior shown in dairy cows which push the nose firmly and directly onto barn objects. Little is known about the reasons why this behavior takes place, only a previous investigation related the performing of this behavior with a decrease on parasympathetic activity. The main task of this study is to investigate whether this previous results may be confirmed in terms of heart rate variability (HRV) during resting, waiting and milking, and to get a global idea about how the barn environment influence the performing of nose pressing in terms of behavior. 7 cows identified as exhibiting nose pressing and 7 control animals were observed in the barn and during the milking procedures along fifteen days. They were monitored with heart rate recorders POLAR® RS800CX and the lying and standing bouts were evaluated using HOBO Pendant® G Data Logger UA-004-64. The behaviors observed in the barn did not differ from NP to control animals; however, there were a higher variability in the first group. During lying, there was not found any difference between groups and, during waiting and milking, NP animals showed a significant lower HR than control cows. The performance of NP behavior during milking showed lower values of RMSSD, STD RR and STD HR than when NP was not showed; i.e. higher parasympathetic activity observed while NP took place. In brief, it seems that NP animals did not trigger relaxing systemic changes by nose pressing. In addition, the great variability of the behavior observations and the intermittence of the performing of nose pressing might prove that nose pressing is a transitory behavior.

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INTRODUCTION

The introduction shows the reader the contextualization of this work; it is focused to expose the hypothesis to be solved and provides the minimum theoretical basis for understanding the work done.

CONTEXTUALIZATION

European consumers are very sensitive to animal welfare, not only due to purely ethical concerns, but also because of a “food quality concept”. They expect that animal products, especially food, respect the animal’s needs. This consumer demand for welfare-friendly animal production has provoked the introduction of regulations and action plans by the European Commission. The main purpose is to regulate, improve and guarantee the protection and welfare of animals (European Comission 2012). Starting on these minimum legal requirements which act to prevent damage and suffering from the animals, voluntary welfare assessments appeared in order to evaluate different levels of welfare state that goes beyond the minimum requirements. In other words, these welfare protocols establish a sorting of animal system procedures which rank from where basic models are implement till systems where even higher levels are practiced (Welfare Quality® 2009). However, new welfare assessment protocols provide an additional tool for the valuation of animal welfare but they do not replace any existing legal standard. In this way, whether the application of any element of the protocol conflicts with the current legal framework, the second should have always priority (Welfare Quality® 2009).

The study of animal welfare has experienced an exponential increasing during the last decades, causing a great impact on the way of animals’ handling, housing technologies and productivity. In high producing dairy cattle, milking systems and housing technologies are main factors in determining animal welfare just like stress evaluation; for this reason, stress assessment has become in a large open field to study. There are behaviours in dairy cattle that have been related to animals under the influence of stressful patterns; e.g. modified sexual behaviour (von Borell, Dobson & Prunier 2007). However, there are still some conducts that are not much known about them; nose pressing behaviour is the perfect example.

NOSE PRESSING BEHAVIOUR

Nose pressing can be described as a behaviour shown by cows in various circumstances. Cows push the nose firmly and directly onto surfaces of the barn during some time as seen in Fig. 1. It may occur in different areas of the barn including the milking parlour and the structures the cows press on may also be different, e.g. feeding racks, water troughs, posts etc.

This behaviour has not been deeply studied; moreover, there is no consistent bibliography to explain the underlying causes or the effects the behaviour may have on the animas. On the contrary, nose pressing events are not difficult to observe in the barn or also during milking procedures. Only (Munksgaard & Simonsen 1996) and (Wierenga & Hopster 1982) have mentioned this behaviour on their respective researches; however, they also included leaning with the forehead against barn equipment as nose pressing behaviour.

Fig. 1. Austrian Fleckvieh cow performing nose pressing behaviour onto a feeding rack rod

Gutmann et al. (2013) investigated nose pressing behaviour in dairy cows in a thorough way by comparing cows exhibiting nose pressing behaviour (NP cows) in the milking parlour with control cows in terms of basic activity and heart rate variability (see chapter Heart rate variability for explanations) during resting, waiting and milking. They obtained that NP cows showed a lower percentage of low frequency spectrum during resting as compared to control animals. Likewise, during waiting before milking the heart rate variability pattern of nose pressing animals showed a higher sympathetic activity. When NP animals showed nose pressing in the milking parlour parasympathetic activity increased. The authors related the behaviour with a possible response to stressor factors and they concluded that nose pressing animals were able to unchain relaxing procedures when showing nose pressing behaviour. Following the criteria and methods previously shown, the main purpose of this study is to confirm the results obtained.

STRESS IN DAIRY COWS

The concept of stress has a key role in animal welfare and it gains importance when assessing welfare in dairy cows. The word “stress” has become very usual in people’s day-to-day conversations; however, the “concept of stress” has a different meaning when faced to animal welfare. It might be defined as the mechanism that leads the individual to be adapted to the surroundings where the animal lives (Farm Animal Welfare Education Centre 2013). The stressor, defined as the agent who triggers the stress response, has an impact on the individual which depends on the emotional state of the animal, the genetic characteristics, previous experiences that the animal faced and how the individual is able to control the situation (Fig. 2). The responses animals perform when facing a stressor may differ, however it is possible to find a common pattern. Generally, normal behaviours observed in non-stressed animals may increase or decrease in individuals; e.g. vocalizations, urinations and defecations, allogrooming and/or movement. Similarly, stressed animals develop altered physiological behaviours as sexual behaviour or lying-standing activity (von Borell et al. 2007). Stereotypical actions are observed too; they are defined as those behaviours repetitive, invariable and without a specific biological function. Despite the fact that parasites or malnutrition infer in the appearance of these behaviours, stereotypical action may be caused as a response to an adaptation due to suffering or frustration as well.

On the other hand, factors which are interpreted as stressor agents are gathered in four different categories. Firstly, interactions between animals are considered as a part of the contacts between the individual and its environment, in the case of intensive farming, with its social surrounding. Every factor which hinders the integration of the individual actions in the society or delays the organization into a hierarchy is able to be considered as a potential stressor (Rodero Serrano 2012); e.g. social isolation, overcrowding and mixture of stranger animals.

Similarly, in-barn environmental conditions as temperature, humidity, ventilation or noise are needed to take into account in order to assess stress and, as a consequence, dairy cows welfare. In the same way, the intensity of stimuli must be considered. Dairy cows must be bred within an environment which offers the animal the precise amount of stimuli, otherwise, an excess might provoke behavioural disorders and loses of productivity (Rodero Serrano 2012). Monotonous environments are injurious and may result in anomalous behaviours such as stereotypes.

Thirdly, cow-human interactions are considered as stressors, and the consequences of these actions may vary from causing simple loses of time in handling, to potential accidents focused on the animal or the handler. Most of the negative effects of this interaction are related with the fear a cow might develop to human presence; as a consequence, dairy cows may show a fight or flee response against these stress sources (Farm Animal Welfare Education Centre 2013).

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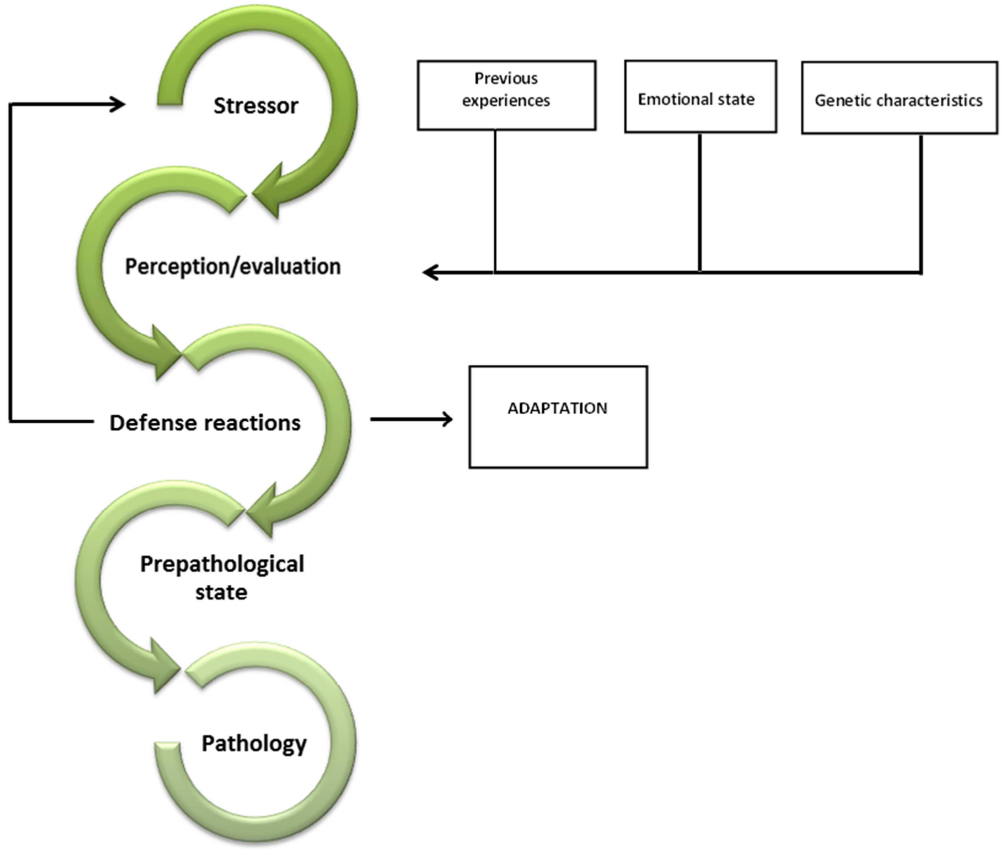

Fig. 2. Model of stress reaction performed by animals (modified from (Rushen et al. 2008)). The response that animals develop to deal with a single stressor depends on its previous experiences. When the animal consumes too much of the animal's resources a pre-pathological stress may occur, and if it is prolonged, it can result into pathology.

Finally, physical pain or illness contributes to the emergence of distress effects; however, poor health may be considered not only as a stressor factor but also as a consequence of stress by itself. In this way, the influence of stressor factors may affect the cow's immune system, so that, pathogenic agents increases the chances to attack the individual leading into the development of new sicknesses.

HEART RATE VARIABILITY

FROM HEART RATE TO HEART RATE VARIABILITY

The heart rate (HR) activity is regulated by the sinoatrial node (SN) that responds to the signals sent through the sympathetic (SNS) and parasympathetic nervous system (PNS). The SNS increases the HR and the response is slow; on the contrary, the PNS modulates the decrease of HR and its response is faster (Tarvainen & Niskanen 2008). As it is exposed in the bibliography, the differences between the release times of the sympathetic and vagal regulators result in new differences in response times (von Borell et al. 2007).

The continuous modulation of the autonomic nervous system (ANS) causes variations in the cardiac activity and the variation in the inter-beat interval (IBI) is described as heart rate variability (HRV). The different frequencies at which the heart rate oscillates can be divided into different spectral bands. First of all, the high frequency band (HF), regulated by the vagus, is a consistent measure of the PSN activity (Porges 1995). At the same time, breathing is the main procedure that impacts the fast rate in the HF range of the HRV.

Another component of the HRV is the low frequency (LF) band, which is mainly associated with the so-called Mayer waves of the blood pressure (Berntson et al. 1997). It is not completely agreed that the LF band has a parasympathetic or sympathetic origin; however, as Houle, et al. (1999) mentioned, the interaction of sympathetic and vagal responses lead into the LF component. Finally, the very low frequency (VLF) band is related to thermoregulation and, similarly to the LF band, it is not totally clear whether its physiological effect has relevance in cattle (Mohr, Langbein & Nürnberg 2002), (Hagen et al. 2005).

Changes in HRV have been carried out by new frequency-modeling approaches which assign the variability of IBIs to the spectral bands exposed; in addition, more informative parameters are included in this sort of analysis. They are time-domain measures and the most instructive HRV variable is the root mean square of successive differences (RMSSD) which was thought so as to evaluate the vagal activity (von Borell et al. 2007). On the other hand, the standard deviation of the normal-to-normal intervals (SDNN or STDRR) reflects the long term variability over 24 hour periods (von Borell et al. 2007).

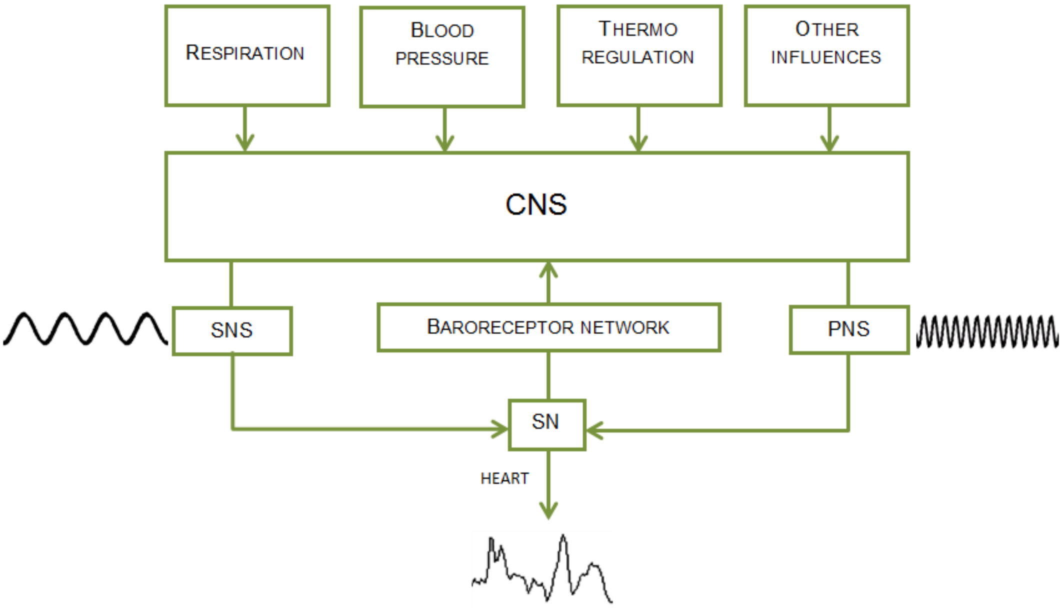

Fig. 3. Simplified diagram of the HRV formation and the primary nervous system between the brain and heart. Based on von Borell, et al., 2007 and (McGraty, Tiller & Atkinson 1996)

HEART RATE VARIABILITY AS A STRESS INDICATOR

During the last decades, some researchers have studied the use of HR and HRV measurements as a measure of stress. It has been shown that HRV is a good indicator of ANS activity related to stress (von Borell et al. 2007); for this reason, the number of studies that use HRV parameters for indicating stress has increased.

It is known that stress has a close relation with the activity of the SNS; therefore, it is easily understood that the ratio between HF and LF power can be used as an indicator of sympathetic activity and, consequently, as a stress indicator (von Borell et al. 2007). This parameter seems to be one key point for analyzing stress in cattle; similarly, high RMSSD values reflects high parasympathetic activity and, therefore, low stress level.

As it has been previously presented, stress can be related to some different situations in day-to-day life of the cows. For example, the effect of pathological disorders and its relation with the presence of stress in dairy cattle has been investigated. Some studies have focused on analyzing the effect of these disorders on HRV. They revealed that there were changes in the activity of the ANS related with infectious diseases (Kovács et al. 2014); e.g. the predominance of vagal tone when increasing RMSSD (Pomfrett et al. 2004) in cows positive for bovine spongiform encephalopathy. In the same way, events that evoke painful situations on the animals can provoke the increase of HR when the animals are re-facing those circumstances; e.g. disbudding, surgery (Kovács et al. 2014). In other words, previous experiences may change the reaction in animals' physiology when they are brought into the same situation again.

Fearful situations may occasion variations on cardiac activity and, as a result, they may be examined as stress sources. This topic has been reiteratively studied and related to routine handling and management procedures. For example, there was shown that HR is affected depending on the way that cows are handled during milking (Rushen, Passillé de AM & Munksgaard 1999). In this study, cows that were able to distinguish between adverse and advantageous handlers were more affected by stress than those animals that were not able to recognize them. And eventually, milking procedures can provoke emotional changes in dairy cattle. Contradictory results have arisen from different studies, so that, "*it seems that the relationship between HR and HRV reactivity to milking is extremely complex"* (Kovács et al. 2014).

For this reason, in the next chapters is analyzed the idea that milking is stressful for dairy cows by the observation of the milking behaviour related. Similarly, the relations between animals of the same herd might suppose another stress source and, as a consequence, it is reported as well.

SOCIAL RELATIONS IN THE HERD

In the herd the relations between individuals may be assessed by different indicators. To begin with, the concept of social dominance is defined as *"a priority access to an approach situation (e.g. food) or away from an avoidance situation (e.g. frightening or painful condition/environment) that one animal has over another"* (van Kreveld 1970). However, it is described that social dominance can be defined by three levels (Langbein & Puppe 2004). Firstly, the starting level of analysis is the dyad as a pair, and it is studied the relation of dominance between both animals. In the second place, the complete group of animals is examined as a single state. The dominance relations seem to be converted in a linear hierarchy as it is exposed in the bibliography (Val-Laillet et al. 2008). Finally, in the third group of analysis are gathered and quantified the "dominance indices". By these calculations, it is described the involvement of all animals in the dominance ranking exposed at the second level.

In other words, animals are less or more prosperous than others at obtaining access to resources as feeding, water or lying places and, those which are low-ranking animals, will have to be more active in order to obtain these incomes. In the same way, they must tolerate or avoid activity when high-ranking or dominant animals are active (Galindo & Broom 2000). This sort of pressure may be understood as a source of stress for low ranking animals and, in this way, it might have an influence over the performance of nose pressing behaviour.

Fig. 4. Two pairs of dairy cows performing allogrooming

On the other hand, some authors consider allogrooming[1] as an indicator of friendship in animals; for this reason, it is believed that social licking has other roles in cattle relations apart from maternal care and reproductive behaviour (Laister et al. 2007). Grooming (Fig. 4) may reinforce inter-individual bonds and it can be understood as a pleasant experience while some animals are receiving this behaviour. However, there are not strong evidences that social licking has a positive effect on animal status. Boissy, et al., 2007 contemplate that for low-ranking cows, performing allogroming could become as a stressfull pattern whether the passive cow is a high-hierarchized animal. For this reasons, the performance of allogrooming in dairy cattle might be lincked with nose pressing in some manner.

Finally, the relations between individuals can be assessed by the analysis of the avoidance from individuals to others (Langbein & Puppe 2004). In this way, those low-hierarchized cows might elude to remain next to a high ranking animal and, whether nose pressing behaviour was linked to low-ranked individuals, show nose pressing behaviour..

One of the purposes of the study developed in the next pages is to relate social relations in dairy cows, which may be evaluated by dominance hierarchy and allogrooming analysis, with the performing of nose pressing behaviour.

[1] Allogrooming or social licking describes the interaction between two animals characterized by repetitive contacts with the tongue and usually directed to the head, neck or forequarter.

OBJECTIVES AND RESEARCH QUESTIONS

The main objective of the study is to confirm or refute the results obtained by Gutmann, et al. (2013), through answering and interpreting the following research questions:

Do cows that have been identified as exhibiting nose pressing show differences in HRV during resting, waiting and milking times?

Do animals trigger relaxating systemic changes when they perform nose pressing?

Moreover, this report is focused on widen the knowledge on nose pressing; so that, the succeeding questions are proposed to be solved:

Is nose pressing behavior only shown during milking procedures?

Do individuals that have been identified as exhibiting nose pressing belong to low–herarchized category?

Is allogrooming related to nose pressing performance?

Do total lying time as well as number and duration of lying bouts differ between nose pressing cows and other animals?

METHODOLOGY

The methodology is mainly structured in five chapters. To start with, in the first chapter is gather different information concerning the location and duration of the study and how the cows were selected into the two groups of research: nose pressing and control.

During the complete observation period the animals were equipped with heart rate monitors. In the second chapter, this is described in detail.

Thirdly, live observations in the barn are described and, in different sections, are defined the four sorts of behaviours observed: nose pressing bouts, frustrating events, licking habits and social interactions.

The fourth chapter contains information about the video recordings taken during the milking phases. As a result, nose pressing bouts and behaviour during milking are included in this section.

Finally, lying and standing phases is analyzed. This fifth chapter is focused on how data is taken and how it is managed.

DESCRIPTION OF THE INVESTIGATION

LOCATION AND FARM DESCRIPTION

The study was carried out at the dairy teaching and research farm of the University of Veterinary Medicine, Vienna. This farm is located in the state of Lower Austria at the village of Kremesberg, about 45 kilometers south-wet of Vienna.

The dairy herd is housed in a 750m^2 barn equipped with 20m wide feed bunk, 6 water troughs distributed along the barn and cubicles for all the cows. Besides, two automatic dispensers that supply the animals with concentrates according to the individual milk yield are installed inside the barn. The outside run covers an area of 200m^2 and it has 2 automatic brushes. The barn connects to the milking parlour, where 8 cows can be milked at the same time in an auto-tandem milking parlour (Fig. 5).

At this farm, cows are milked twice daily. The first one is programmed from 5 am to 7 am and the second is planned from 4 pm to 6 pm. If cows suffer from mastitis, they are milked separately following a standard procedures protocol in order to avoid cross contamination of healthy cows.

DURATION

The observation period took place during four weeks, three days per week, from February 14th until March 8th. As previously described, the farm is a teaching facility, and due to the fact that the observations should be carried out under largely undisturbed conditions, the days selected for the observations were Friday, Saturday and Sunday. However, data collection period had to be extended one extra week because of data related with social hierarchy was not enough to be analyzed. Table 1 collects the final schedule.

Table 1. Final schedule for the data collection in the barn and milking parlour

<table>
<tr><th>Week</th><th colspan="5">Data collection</th></tr>
<tr><td>1</td><td rowspan="5">Live observations</td><td rowspan="4">Video recordings</td><td rowspan="4">HR monitors</td><td rowspan="4">Lying-Standing analysis</td><td></td></tr>
<tr><td>2</td><td></td></tr>
<tr><td>3</td><td rowspan="2">Sector analysis</td></tr>
<tr><td>4</td></tr>
<tr><td>Extra</td><td></td><td></td><td></td><td></td></tr>
</table>

COW SELECTION

The flock was formed in total by 71 dairy cows: 50 Austrian Fleckvieh, 11 Holstein Friesian and 10 Brown Swiss. Due to the fact that all the animals were in the same conditions for the research, the complete flock was taken into account to develop the research.

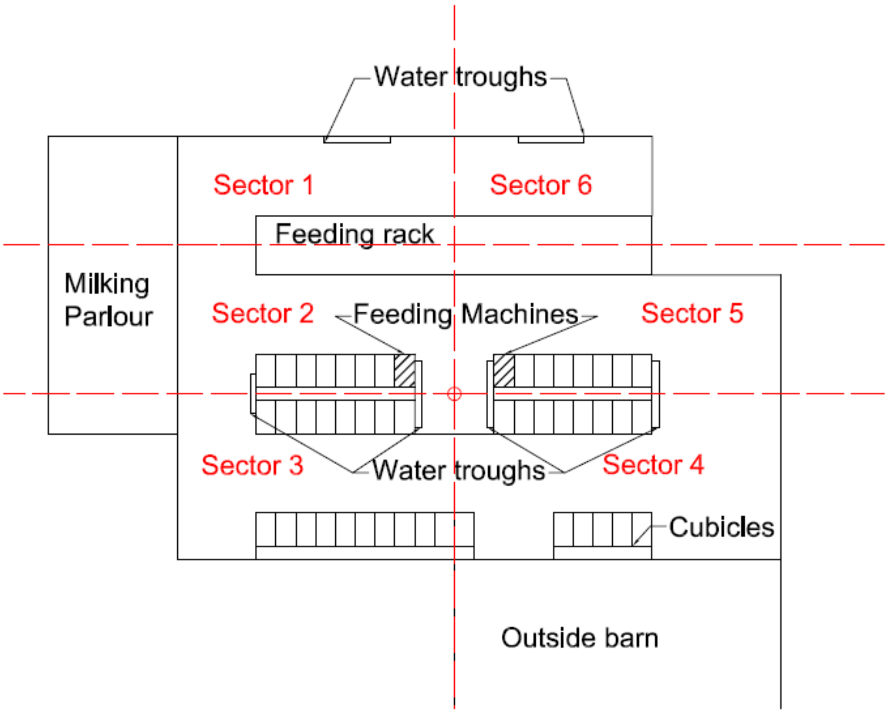

Fig. 5. Barn diagram. Dairy teaching and research farm of the University of Veterinary Medicine Vienna at Kremesberg

For this investigation, it was needed to form two groups of study with the same characteristics; for this reason, three milking phases per cow were observed in order to define the groups. One week before the beginning of data collection, the complete herd was observed and, those cows which showed nose pressing behaviour were pre-selected. For each pre-selected nose pressing cow, control animals of the same breed and similar age and stage of lactation were identified. Finally, 20 cows were selected and divided in the two groups specified in Table 2.

Table 2. The number, name, ear-Tag number, date of birth, expected date of calving and breed of the cows gathered in the nose pressing group.

Group	Number	Name	Ear-Tag Number	Date of birth	Expected Date of Calving	Breed
Nose Pressing	1	LAMPI	AT000000318316	25/05/2008	20/07/2014	AF[2]
	11	HENNI	AT000768667616	13/12/2008		AF
	13	NAPOLI	AT000923921417	16/08/2010		AF
	14	SENTA	AT000579706317	23/03/2010	17/06/2014	BS[3]
	19	ROLINA	AT000827461272	28/02/2004		BS
	27	GRÄFIN	AT000833910118	13/11/2010		AF
	29	HELENA	AT000624630507	22/02/2005	18/05/2014	AF
	64	LATOYA	AT000227644217	26/07/2009		AF
	41	GLORIETE	AT000064550209	27/09/2005		AF
	74	LOLA	AT000834056709	03/10/2006	08/07/2014	AF
Control	84	LUCIA	AT000000313716	10/05/2008	17/09/2014	AF
	44	MARIKA	AT000000298916	04/04/2008		AF
	76	ZUCKERL	AT000923920317	14/08/2010		AF
	34	RIO	AT000163657319	10/05/2011	25/06/2014	BS
	59	BLANKA	AT000094645614	17/10/2006		BS
	23	BRIMEL	AT000923932717	04/11/2010		AF
	31	LOREN	AT000624634907	27/02/2005	15/08/2014	AF
	7	LAMBADA	AT000227643117	26/07/2009		AF
	35	NORIKA	AT000064524909	15/06/2005		AF
	69	BETINA	AT000182641814	14/01/2007	08/07/2014	AF

CHANGES MADE IN COW GROUPS

THE TWO GROUPS OF COWS HAD TO BE MODIFIED DUE TO DIFFERENT REASONS. COWS 29, 64, 41 AND 74, WHICH WERE GATHERED IN THE NOSE PRESSING GROUP AT THE BEGINNING OF THE RESEARCH, DID NOT SHOW NOSE PRESSING BEHAVIOUR DURING THE DATA COLLECTION PERIOD. CONVERSELY, COWS 23 AND 69 WERE GROUPED AS CONTROL ANIMALS BUT THEY SHOWED THE BEHAVIOUR SEVERAL TIMES. IN ADDITION, NOSE PRESSING COW 14 SHOWED NOSE PRESSING IN THE BARN BUT IT DID NOT SHOW THE BEHAVIOUR DURING MILKING. ON THE OTHER HAND, HEART RATE DATA FILES FROM COWS 31 AND 59 CONTAINED TOO MANY ERRORS. DUE TO THE FACTS EXPOSED, IT WAS

[2] Austrian Fleckvieh
[3] Brown Suiss

DECIDED TO REASSEMBLE THE COWS FOLLOWING THE SAME CRITERIA MENTIONED IN THE CHAPTER

Cow selection. Cows 29, 64 and 41 were changed from the nose pressing group to the control group; on the contrary, cows 23 and 69 were changed from the control group to the nose pressing group. Cow 14 was excluded from the study due to its behaviour. Finally, the groups were formed by seven cows each as specified in Table 3 referred to as Nose Pressing and Control groups in the following sections.

Table 3. The number, name, ear-Tag number, date of birth, expected date of calving and breed of the cows gathered in the reassembled groups.

Groups	Number	Name	Ear-Tag Number	Date of birth	Last Calving Date	Breed
Nose Pressing	1	LAMPI	AT000000318316	25/05/2008	11/07/2013	AF
	11	HENNI	AT000768667616	13/12/2008	25/08/2013	AF
	13	NAPOLI	AT000923921417	16/08/2010	24/01/2014	AF
	19	ROLINA	AT000827461272	28/02/2004	31/05/2013	BS
	23	BRIMEL	AT000923932717	04/11/2010	22/12/2013	AF
	27	GRÄFIN	AT000833910118	13/11/2010	09/12/2013	AF
	69	BETINA	AT000182641814	14/01/2007	13/07/2013	AF
Control	84	LUCIA	AT000000313716	10/05/2008	01/08/2013	AF
	35	NORIKA	AT000064524909	15/06/2005	06/09/2013	AF
	7	LAMBADA	AT000227643117	26/07/2009	17/12/2013	AF
	34	RIO	AT000163657319	10/05/2011	26/06/2013	BS
	41	GLORIETE	AT000064550209	27/09/2005	30/10/2013	AF
	64	LATOYA	AT000227644217	26/07/2009	24/11/2013	AF
	29	HELENA	AT000624630507	22/02/2005	03/06/2013	AF

It was checked the tendency of the productivity observed in both groups and, as seen in Fig. 6 and Fig. 7, both are similar and, so that, they are comparable.

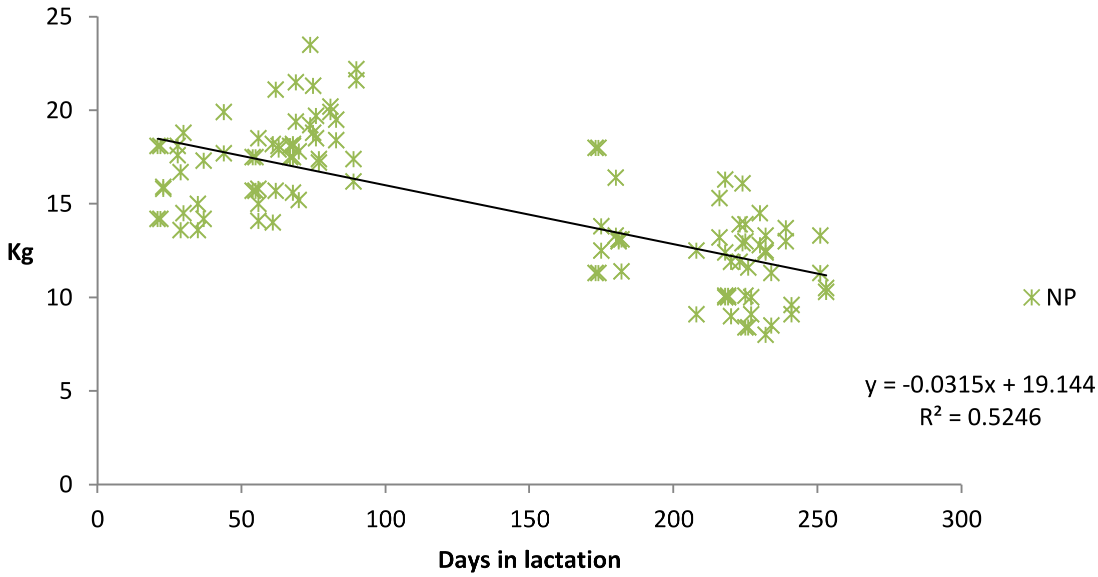

Fig. 6 Nose pressing group production chart. Productivity (kg) per animal and day of lactation

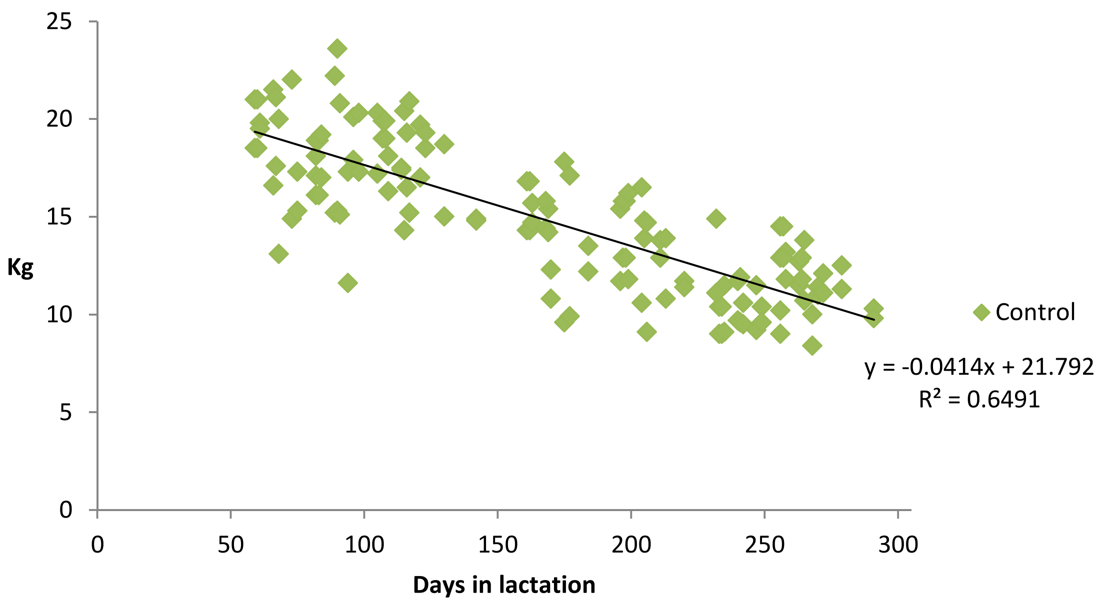

Fig. 7 Control group production chart. Productivity (kg) per animal and day of lactation

VIDEO RECORDINGS

Throughout the observation period, milking times were video recorded. For this purpose, eight cameras were installed inside the milking parlour and connected to a digital receiver. Recordings were stored inside a hard drive and backed up every week. In this way, those milking phases of each focus animal which heart rate recordings were accepted (see chapter below "Heart rate analysis: Error correction") were watched emphasizing on nose pressing bouts and behaviour during milking.

First, the start and end time of the nose pressing bouts was recorded. Thereafter, the observer divided the complete milking phase into 30 second sub-periods. All sub-periods were then allocated to a category related to the duration of nose pressing according to Table 4.

Table 4. Classification of 30 second sub-periods according to the duration of nose pressing within each 30s bout

Category	Description
1	NP is observed; duration ≥ 30s
2	NP is observed; duration ≥ 15s
3	NP is observed; duration < 15s
4	NP is not observed.

Additionally, three further behaviours during milking were recorded: kicking, stepping and provision of food. Kicking behaviour is defined as a cow lifting a foot and moving it forward (Kick) or lifting one hind in order to hit the milking cluster or the handler (Kick-Kick). Conversely, stepping behaviour describes a cow shifting its body weight (Wenzel, Schönreiter-Fischer & Unshelm 2003). Finally, the provision of food in terms of the milker putting some concentrate mesh into the trough of the milking stall in order to encourage the cow to enter or to calm it during the milking procedure was recorded.

HEART RATE ANALYSIS

HEART RATE MONITORS

All the animals selected for the study were equipped with heart rate monitors POLAR® RS800CX. This equipment was formed by one monitor and one pair of sensors. In order to fix the sensors to the skin of the animal, neoprene belts were used that covered the complete set. Moreover, and to guarantee a perfect signal reception, wet sponges were inserted between each sensor and the skin of the animals. It was a key point to maintain the sponges wet; for this reason, they had to be checked twice a day. The monitor had to be every time close enough to the sensor in order to avoid signal reception problems. Each monitor was kept inside a pocket and attached to the collar.

Every Friday after the morning milking, nose pressing and control cows were provided with the belts which covered and protected the monitors and POLAR® RS800CX devices. Data from monitors was downloaded to a computer every 24 hours and saved through Polar® ProTrainer Equine Edition software. For this reason, it was accorded to download data every morning after the first milking and reinstall them again as soon as data was saved. Throughout the data collection time, each animal did not wear the same monitor so as to avoid accumulating possible errors. However, every monitor was matched with its sensors.

Therefore, the POLAR® RS800CX monitors were uninterruptedly recording from Friday morning until Sunday morning, throughout four weeks, except for the time needed for the downloading procedures. A maximum number sixteen milking phases was recorded per focus cow (nose pressing and control).

ERROR CORRECTION

For HRV analysis, errors in the dataset have to be corrected. Polar® ProTrainer Equine Edition software provides a possibility to identify and remove such errors, which is also used in this study. Five minute windows of the heart rate graphic were used and the built-in correction mode was applied if the error rate was less than 5% (von Borell et al. 2007). Those phases which include higher percentage of error were not corrected and excluded from the analysis.

HEART RATE VARIABILITY ANALYSIS

The analysis of the data sets resulting from POLAR® RS800CX devices and corrected by Polar® ProTrainer Equine Edition software was made using Kubios HRV software. However, previous to this examination, three stages were defined.

a) **Waiting.** Period of ten minutes before the cow entered the milking stall.
b) **Milking.** Time that each cow spent inside the milking stall.

c) **Lying.** Time that cows spent in a horizontal position on the surface of the cubicles. The first fifteen minutes and the last ten were not included in the calculations.

These episodes were analyzed following the instructions provided by Kovács, et al. (2013) and Tarvainen, et al. (2008), which are summarized in Table 5. The HRV parameters used in the present study are given in Table 6.

Table 5. Correlation between waiting, milking and lying stage and the values needed to perform the HRV analysis by Kubios HRV Software.

<table>
<tr><th>Stage</th><th>Length of the sample</th><th>Method</th><th>Lambda</th><th colspan="2">Frequency bands (Hz)</th></tr>
<tr><td>Waiting</td><td>30(s)</td><td rowspan="3">Smoothness priors</td><td rowspan="3">500</td><td rowspan="3">VLF
LF
HF</td><td rowspan="3">0-0.04
0.04-0.25
0.25-0.58</td></tr>
<tr><td>Milking</td><td>30(s)</td></tr>
<tr><td>Lying</td><td>300(s)</td></tr>
</table>

Table 6. Definitions of the values proposed in order to analyze the HRV.

STD RR (ms)	Standard deviation of RR intervals
Mean HR (1/min)	The mean heart rate
STD HR (1/min)	Standard deviation of instantaneous heart rate values
RMSSD (ms)	Square root of the mean squared differences between successive RR intervals
LF (%)	Relative power of low frequency band
HF (%)	Relative power of high frequency band
LF/HF ratio	Ratio between LF and HF band powers

HRV parameters were evaluated using mixed models for repeated measures with the interaction between day and period as repeated factor and cow as random factor. This model was calculated in SAS 9.2 (proc. mixed, repeated day * period, subject cow) and the statistical significance selected was α=0.05. The model was focused on contrasting the nose pressing and control groups during the waiting time before milking; besides, it compared the HRV values during the milking time in three different cases. Firstly, both nose pressing and control animals were matched up; secondly, nose pressing bouts labeled as category 1 (Table 4) were compared with bouts labeled as category 4.

Finally, nose pressing bouts 1 and 2 were grouped and matched up against categories 3 and 4.

On the third place, the difference between HRV parameters obtained from milking and waiting time was analyzed. Again, nose pressing and control groups were compared; secondly, labels 1 versus label 4 and, finally, labels 1 and 2 against labels 3 and 4.

Lying HRV parameters were analyzed through a Student's t-test (two tails, equal sample size and equal variances) using the same statistical significance as in previous calculations (α=0.05).

LIVE OBSERVATIONS

Observations of the fourteen selected animals took place inside the barn excluding the milking parlour. Observations were carried out in six virtual sectors and lasted for 15 min each. Continuous focal animal behaviour sampling was used to record the behaviours explained below. At the start of each observation period, all focal animals in the sector were noted. Observations took place on in total fifteen days. They were carried out between 07:30 and 19:30 on Fridays and Saturdays and between 07:30 and 14:00 on Sundays. The actual time available for observations was not equal over the fifteen days of data collection due external influences such as cow weighing or cubicle cleaning. Observations were distributed in order not to concentrate the data set in one time band; the total time observed per cow is shown in Table 7.

Table 7. Collection of cow groups and time observed per animal

Control	Cow	Time observed (h)
NP	1	13.1
	11	15.9
	13	11.3
	19	14.2
	23	11.8
	27	14.3
	69	14.4
Control	7	15.3
	29	12.4
	34	14.2
	35	12.6
	41	14.3
	64	14.5
	84	11.8

NOSE PRESSING HABITS

The cow pushes the nose firmly and directly onto surfaces of the barn. The minimum length of a nose pressing bout was 15 seconds.

POTENTIALLY FRUSTRATING EVENTS

Frustrating events were defined as situations that presumably cause stress due to not meeting the animal expectations. For this purpose, events like showing several failed attempts to rise from the cubicles or not getting concentrates from the feed dispensers were included. The latter event was usually combined with aggressive kicking against the machine and vocalizations.

SECTOR OBSERVATION

Lastly, it was observed which cows were in each sector. The objective of these observations was to know whether there was any kind of social preference between the cows; for this reason, it was noted which cow was in which sector. These data entries were collected twenty times during six days and taking into account different time periods of the day.

It was calculated the percentage of times (scans) that each focal animal was observed with any other animal of the herd in the same sector. The maximum percentage obtained was 57%. Therefore data were classified in four categories (0-5, 5-20, 20-40, 40-60 %).

In order to analyzed the data set obtained, values were evaluated through a Chi-square test (α=0.05).

STEREOTYPIC LICKING HABITS

This group basically focused on repetitive oral behaviours in cattle. The most common pattern in cattle is known as tongue-rolling and it consists of a continuous rolling and stretching of the tongue inside or outside of the mouth. It may be easily identified by the observer and it is sometimes accompanied by head nodding (Redbo 1998).

SOCIAL INTERACTIONS

For this group of behaviours, the following behaviours were defined:

a. **Displacement.** A cow hits with its head or parts of the body against another animal and causes the other animal to move away for at least on cow width (movement to the side) or half a cow length (longitudinal movement).
b. **Allogrooming.** Repetitive contact with the tongue onto the skin of another animal.
c. **Head-play.** All playful behaviours involving a direct contact of the heads of two animals. Sometimes, it is not easy to distinguish between play and aggression (Rushen et al. 2008); the observer had to decide if the animals gave the impression they were playing rather than fighting.
d. **Mounting.** A cow ascending another cow with both front legs.

While observing the four groups of behaviours, the observer had to indicate whether the focused animal was performing the behaviour or, on the contrary, it was receiving the action.

CALCULATION OF BEHAVIOURAL INDICES

For each animal, four behavioural indices were calculated. First of all, displacement indices were considered in order to define social competitive interactions inside the herd.

1. *Galindo-Broom Index*: This index is based on the proportion of displacements that an animal initiated compared to the number of displacement events that the same animal experienced (Galindo & Broom 2000).

$$Galindo - Broom\ Index = \frac{number\ of\ times\ the\ individual\ displaces\ any\ cow}{\begin{array}{c} number\ of\ times\ the\ individual\ displaces\ any\ cow\ + \\ number\ of\ times\ the\ individual\ has\ been\ displaced \end{array}}$$

Eq. 1

2. *Mendl Index*: It is based on agonistic interactions (Mendl, Zanella & Broom 1992). For this index, one single displacement is enough to be counted as a cow able to displace.

$$Mendl\ Index = \frac{number\ of\ cows\ that\ an\ indivdual\ is\ able\ to\ displace}{\begin{array}{c} number\ of\ cows\ that\ an\ indivdual\ is\ able\ to\ displace + \\ number\ of\ cows\ that\ are\ able\ to\ displace\ the\ individual \end{array}}$$

Eq. 2

Finally, behavioural indices based on allogrooming were calculated. In the same way, the first index was based on the proportion of licking events that an animal initiated whereas the second one was based on agonistic interactions.

$$Index\ 1 = \frac{number\ of\ times\ the\ individual\ licks\ any\ cow}{\begin{array}{c} number\ of\ times\ the\ individual\ licks\ any\ cow\ + \\ number\ of\ times\ the\ individual\ has\ been\ licked \end{array}}$$

Eq. 3

$$Index\ 2 = \frac{number\ of\ cows\ that\ an\ indivdual\ is\ able\ to\ lick}{\begin{array}{c} number\ of\ cows\ that\ an\ indivdual\ is\ able\ to\ lick + \\ number\ of\ cows\ that\ are\ able\ to\ lick\ the\ individual \end{array}}$$

Eq. 4

LYING AND STANDING PHASES

Knowing the lying and standing phases was a key point in this research. Therefore, each focal animal was equipped with a HOBO Pendant® G Data Logger UA-004-64 (Fig. 8). This equipment can measure acceleration in one, two or three axes. For monitoring standing and lying time, devices were programmed to measure acceleration in two axes (Y and Z). The system was programmed to store values every 30 seconds.

In order to get correct data, it was important to standardize the orientation of the logger attached to the lateral metatarsus of the cow. A SOP provided by University of British Columbia (UBC AWP 2013) was followed in order to establish these basic rules to set the devices and to analyze the data set obtained.

In brief, the logger and the silicone mold were attached using at least two layers of vet-wrap around the lower part of the leg (UBC AWP 2013). The device was placed on the outside part of the right leg as shown in Table 8.

Table 8. Orientation recommended for placing HOBO Loggers on the right hind leg of the animals with the purpose of recording lying-standing phases.

Axes	Orientation
X-axis	Parallel to the ground
Y-axis	Perpendicular to the ground
Z-axis	Parallel to the ground pointing left

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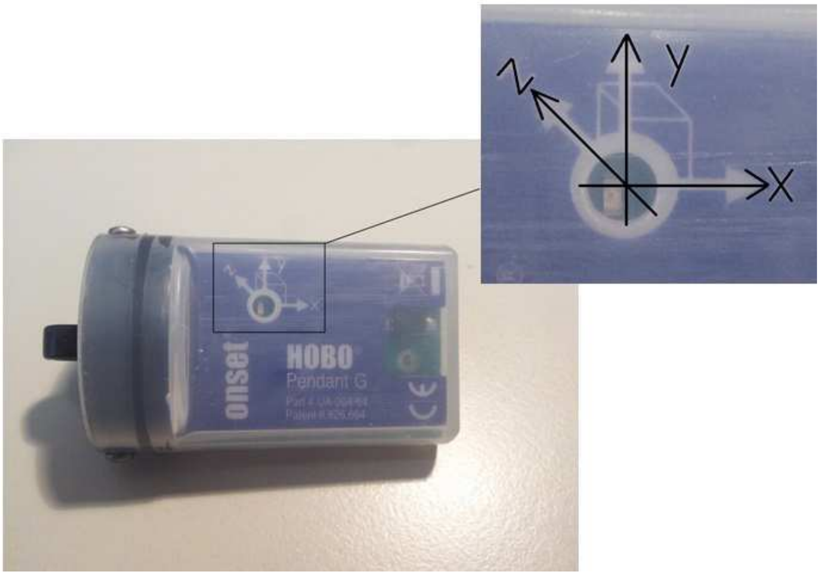

Fig. 8. HOBO Pendant® G Data Logger UA-004-64

SUMMARIZING LYING PHASES USING EXCEL SOFTWARE

To analyze lying behaviour, .txt files originating from HOBOware Lite were imported to Excel software. An Excel macro distinguished between lying and standing phases based on the Y-axis; in this way, the acceleration values obtained during the milking phases were used as reference values in order to differentiate between lying and standing phases. Thirty seconds readings with Y-axis values equal or lower than -0.8g were considered as standing phases. On the contrary, those bouts with Y-axes values higher than -0.8g were set as lying phases. However, it was needed to establish an error correction to remove possible wrong-recorded data; using the same Excel file, those standing or lying bouts with a duration of less than one minute were corrected (Table 9). Through the data set exported from HOBOware Lite those bouts longer than five minutes where included and analyzed by a two sided tails Student's t-test (α=0.05).

Table 9. Analysis of lying phases. Example of error correction carried out by an Excel macro.

Cow	Date	Time, GMT+01:00	Acel Y, g	Acel Z, g	Position	Corrected position
13	28/02/2014	16:51:00	-1	-0.3	Standing	Standing
13	28/02/2014	16:51:30	-1.025	-0.275	Standing	Standing
13	28/02/2014	16:52:00	-1	-0.325	Standing	Standing
13	28/02/2014	16:52:30	-0.725	-0.275	Lying	Standing
13	28/02/2014	16:53:00	-0.975	-0.325	Standing	Standing
13	28/02/2014	16:53:30	-1.2	-0.525	Standing	Standing
13	28/02/2014	16:54:00	-1.025	-0.35	Standing	Standing

RESULTS

This section contains the results obtained during the study. To obtain them the protocols exposed in the chapter Methodology have been followed.

VIDEO OBSERVATIONS

OCCURRENCE OF NOSE PRESSING BEHAVIOUR

The mean duration of nose presser animals was slightly longer than the control group (Table 10). In this way and regarding nose pressing animals, the duration was greater in the mornings than in the afternoon milking. Finally, the mean duration of the nose pressing bouts observed was similar in both periods. Nose pressing cows showed NP during the 34.4% of the milking phases and the percentage increased in the afternoon milking phases.

Table 10. Mean duration of milking and nose pressing bouts observed in the milking parlour over all cows and milking phases analyzed (sample sizes; n_1=60, n_2=62)

		Milking Duration (min)			NP Duration (min)		
Control	**(n_1)**	14.66	±	4.09	0	±	0
NP (n_2)	**Total**	13.61	±	5.38	4.75	±	3.94
	Morning	15.39	±	4.15	4.85	±	3.88
	Afternoon	14.1	±	4.01	4.68	±	4.04

Fig. 9. Austrian Fleckvieh dairy cow performing nose pressing on the edge of the trough during the milking procedure

Regarding the performance of nose pressing (Fig. 9), in Table 11 is observed that the percentage of nose pressing time during the milking phases is greater in the afternoon period; nevertheless, the amount of bouts observed in the mornings is a little superior than in the other interval. Another point is that, it was observed a higher percentage of complete nose pressing milking phases (L1) than those phases where the behaviour is partially presented (L2, L3). However, in the majority of the milking phases the nose pressing performance was not observed. It is needed to mention the high variability of the results obtained.

Table 11. Mean percentage of nose pressing bouts observed in the milking parlour and percentage observed of each label over all milking phases (sample size; n=62)

	NP	NP Morning	NP Afternoon
NP (%)	34.4 ± 28.7	32.2 ± 26.2	36.1 ± 30.8
NP Bouts	3.71 ± 2.46	3.89 ± 2.33	3.57 ± 2.58
L1 (%)	16.0 ± 19.3	14.6 ± 17.4	17.1 ± 20.8
L2 (%)	8.68 ± 6.61	8.68 ± 5.28	8.69 ± 7.54
L3 (%)	11.1 ± 9.45	10.4 ± 8.58	11.7 ± 10.16
L4 (%)	55.9 ± 31.4	57.2 ± 29.3	55.0 ± 33.3

KICKING, STEPPING AND FOOD PROVIDING.

During the milking procedures, the behaviours defined in the page 27 were observed. In Table 12 are gathered the behaviours observed during milking phases and the mean of each behaviour observed. It is not possible to asses that there are differences between the statistical distributions of both groups (NP and control). In the same way, the feed supplying apparently did not suppose a measurable effect on the reduction of the behaviours.

Table 12. Number of step, kick and kick-kick events during the milking phase: means calculated depending on whether feed were provided. F = Fore leg; H = Hind leg.

			NP			Control				
			Mean		**STD**	**Mean**		**STD**	**F**	**p**
Feed Independent	Step	F	9.44	±	6.16	8.44	±	6.00	0.274	0.789
		H	6.17	±	4.33	6.88	±	3.22	0.288	0.778
	Kick		8.86	±	5.18	6.19	±	5.20	1.241	0.238
	Kick-Kick		5.97	±	3.50	5.11	±	6.16	0.335	0.744
Feed was provided	Step	F	9.60	±	6.15	8.88	±	5.17	0.174	0.865
		H	5.84	±	4.47	6.79	±	3.05	0.397	0.698
	Kick		9.63	±	5.09	6.08	±	4.51	1.651	0.125
	Kick-Kick		6.09	±	3.54	3.70	±	2.18	0.922	0.375
Feed was not provided	Step	F	10.0	±	9.32	4.77	±	4.28	1.876	0.085
		H	7.05	±	2.31	5.26	±	3.36	0.635	0.537
	Kick		7.96	±	3.86	7.15	±	6.88	0.246	0.810
	Kick-Kick		4.40	±	2.06	8.68	±	9.22	1.215	0.248

HEART RATE VARIABILITY

WAITING TIME

Table 13 gathers the values obtained from calculations related to the waiting time before milking. There are significant differences between the values acquired during the morning and afternoon milking except for STD RR.

However, the only measure showing a difference between NP and control cows was Mean HR (p=0.001) with higher heart rate in control animals than in NP animals. Finally, there was no significant effect of the interaction group*period, neither referring to waiting time analysis nor the rest of the analyses made. For this reason, below no further reference is made to these interactions.

MILKING TIME

HRV values of nose pressing and control groups during milking (morning and afternoon) are presented in Table 14. Again, there was higher sympathetic activity during the afternoon milking than in the morning (HR, STD HR, STD RR, RMSSD, HF %). Also the effect of "group" during milking was similar to the pattern shown during the waiting time. Only Mean HR showed higher sympathetic activity in the control group than in the nose pressing group.

As previously mentioned in the methodology chapter (Table 4) for the NP cows the complete milking was subdivided into thirty second phases and labeled depending on the presence of nose pressing bouts and their duration. In this way, Table 15 collects data from nose pressing bouts labelled as 1 and 4. In this case, the effect of "period" is less strong, but HR, STD HR and RMSSD still demonstrated higher sympathetic activity in the afternoon. With regard to the effect of nose pressing, STD HR and STD RR intervals were significantly higher when cows shoed continuously nose pressing; similarly, RMSSD was lower in this case.

Finally, categories 1 and 2 were grouped together and compared with categories 3 and 4. HR was higher during the afternoon milking and RMSSD lower (Table 16). The ratio between LF and HF was also higher in the afternoon. When grouping bouts with continuous nose pressing and bouts with at least 50% nose pressing, no significant differences were found for any HRV measure as compared with bouts with a maximum of 50% nose pressing.

Table 13 HRV values during waiting time (10 min) before milking and effects of “group” (nose pressing, control) and “period” (morning, afternoon) as well as the interaction between group and period.

	Group			Period			
HRV variable	**NP**	**Control**	**Effect of "Group"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	***73.0 ± 0.93***	***78.4 ± 0.91***	**F= 16.91; p= 0.001**	***72.3 ± 0.80***	***79.2 ± 0.74***	**F= 70.3; p= 0.000**	F= 0.25; p= 0.874
STD HR (1/min)	*1.35 ± 0.18*	*1.79 ± 0.18*	F= 3.04; p= 0.103	***1.47 ± 0.14***	***1.67 ± 0.13***	**F= 7.17; p= 0.009**	F= 1.88; p= 0.174
STD RR (ms)	*11.0 ± 41.46*	*12.9 ± 1.46*	F= 0.53; p= 0.478	*12.44 ± 1.07*	*11.8 ± 1.05*	F= 1.55; p= 0.216	F= 2.64; p= 0.108
RMSSD (ms)	*6.95 ± 0.76*	*6.80 ± 0.76*	F= 0.02; p= 0.897	***7.58 ± 0.58***	***6.18 ± 0.56***	**F= 12.9; p= 0.001**	F= 0.90; p= 0.347
LF (%)	*66.9 ± 1.75*	*69.7 ± 1.75*	F= 1.26; p= 0.279	***67.4 ± 1.31***	***69.2 ± 1.28***	**F= 5.45; p= 0.022**	F= 0.14; p= 0.737
HF (%)	*8.91 ± 1.18*	*6.22 ± 1.17*	F= 2.62; p= 0.126	***8.64* ± 0.94**	***6.49* ± 0.89**	**F= 7.47; p= 0.007**	F= 0.08; p= 0.778
LF/HF ratio	*35.4 ± 7.58*	*48.2 ± 7.56*	F= 1.42; p= 0.252	***35.6 ± 5.94***	***48.0 ± 5.68***	**F= 7.70; p= 0.007**	F= 2.56; p= 0.113

Table 14. HRV values during milking time and effects of “group” (nose pressing, control) and “period” (morning, afternoon) as well as the interaction between group and period.

	Group			Period			
HRV variable	**NP**	**Control**	**Effect of "Group"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	***73.6 ± 0.46***	***78.4 ± 0.48***	**F= 52.7; p= 0.000**	***72.4 ± 0.42***	***79.5 ± 0.52***	**F= 115; p= 0.000**	F= 0.89; p= 0.350
STD HR (1/min)	*1.31 ± 0.18*	*1.69 ± 0.18*	F= 2.22; p= 0.158	***1.43 ± 0.13***	***1.58 ± 0.13***	**F= 4.50; p= 0.038**	F= 0.16; p= 0.690
STD RR (ms)	*10.8 ± 1.47*	*12.5 ± 1.47*	F= 0.66; p= 0.431	***12.1 ± 1.06***	***11.2 ±1.05***	**F= 5.00; p= 0.028**	F= 1.34; p= 0.249
RMSSD (ms)	*6.87 ± 0.91*	*6.69 ± 0.91*	F= 0.02; p= 0.892	***7.69 ± 0.68***	***5.86 ± 0.67***	**F= 22.3; p= 0.000**	F= 0.11; p= 0.739
LF (%)	*67.8 ± 1.87*	*71.0 ± 1.87*	F= 1.42; p= 0.252	*69.2 ± 1.38*	*69.5 ± 1.35*	F= 0.20; p= 0.657	F= 0.01; p= 0.933
HF (%)	*9.65 ± 1.57*	*6.33 ± 1.56*	F= 2.26; p= 0.155	***9.07* ± 1.18**	***6.91* ± 1.15**	**F= 9.00; p= 0.003**	F= 0.52; p= 0.473
LF/HF ratio	*34.6 ± 8.73*	*47.9 ± 8.71*	F= 1.16; p= 0.298	*37.6 ± 6.58*	*45.0 ± 6.41*	F= 3.29; p= 0.073	F= 2.13; p= 0.148

Table 15. HRV values during milking time and effects of "label" (1: complete nose pressing bout; 4: no nose pressing observed) and "period" (morning, afternoon) as well as the interaction between label and period.

	Label			Period			
HRV variable	**1**	**4**	**Effect of "Label"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	*73.9 ± 1.06*	*74.8 ± 0.98*	F= 0.70; p= 0.405	***71.5 ± 1.06***	***77.2 ± 0.98***	**F= 28.2; p= 0.000**	F= 0.28; p= 0.597
STD HR (1/min)	***1.22 ± 0.21***	***1.46 ± 0.21***	**F= 4.70; p= 0.033**	***1.22 ± 0.21***	***1.47 ± 0.21***	**F= 5.28; p= 0.024**	F= 0.45; p= 0.503
STD RR (ms)	***10.4 ± 1.76***	***11.9 ± 1.74***	**F= 5.35; p= 0.023**	*11.0 ± 1.76*	*11.3 ± 1.74*	F=0.29; p= 0.592	F= 0.18; p= 0.669
RMSSD (ms)	***6.85 ± 1.46***	***8.10 ± 1.44***	**F= 4.98; p= 0.028**	***8.25 ± 1.46***	***6.70 ± 1.44***	**F= 7.46; p= 0.008**	F= 1.44; p= 0.234
LF (%)	*69.2 ± 2.53*	*67.2 ± 2.48*	F= 1.75; p= 0.189	*68.4 ± 2.56*	*68.0 ± 2.47*	F= 0.06; p= 0.785	F= 0.32; p= 0.572
HF (%)	*10.7 ± 2.67*	*10.5 ± 2.64*	F= 0.03; p= 0.869	*11.7 ± 2.68*	*9.54 ± 2.63*	F= 3.27; p= 0.074	F= 0.87; p= 0.354
LF/HF ratio	*31.1 ± 9.79*	*37.5 ± 9.52*	F= 0.92; p= 0.340	*27.7 ± 9.88*	*40.9 ± 9.47*	F=3.75; p= 0.056	F= 0.23; p= 0.635

Table 16. HRV values during milking time and effects of "label" (1: complete nose pressing bout; 2: nose pressing observed >50% of the bout; 3: nose pressing observed <50% of the bout; 4: no nose pressing observed) and "period" (morning, afternoon) as well as the interaction between label and period.

	Label			Period			
HRV variable	**1-2**	**3-4**	**Effect of "Label"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	*73.6 ± 0.93*	*74.7 ± 0.90*	F= 1.168 ; p= 0.282	***71.1 ± 0.96***	***77.15 ± 0.89***	**F= 38.1 ; p= 0.000**	F= 0.04 ; p= 0.842
STD HR (1/min)	*1.23 ± 0.19*	*1.36 ± 0.19*	F= 3.308 ; p= 0.072	***1.15 ± 0.19***	***1.44 ± 0.19***	**F= 15.0 ; p= 0.000**	F= 0.00 ; p= 0.966
STD RR (ms)	*10.5 ± 1.57*	*11.1 ± 1.56*	F= 1.804 ; p= 0.182	*10.5 ± 1.57*	*11.10 ± 1.56*	F= 2.05 ; p= 0.155	F= 0.44 ; p= 0.507
RMSSD (ms)	*6.35 ± 1.06*	*7.06 ± 1.06*	F= 3.711 ; p= 0.057	***7.28 ± 1.07***	***6.13 ± 1.06***	**F= 9.19 ; p= 0.003**	F= 0.02 ; p= 0.889
LF (%)	*67.5 ± 2.22*	*66.0 ± 2.19*	F= 1.273 ; p= 0.262	*65.8 ± 2.26*	*67.50 ± 2.17*	F= 1.36 ; p= 0.247	F= 0.20 ; p= 0.658
HF (%)	*9.03 ± 2.01*	*9.91 ± 2.00*	F= 0.680 ; p= 0.412	*10.5 ± 2.04*	*8.42 ± 1.98*	F= 3.75 ; p= 0.055	F= 0.15 ; p= 0.698
LF/HF ratio	*33.4 ± 9.46*	*34.3 ± 9.36*	F= 0.022 ; p= 0.882	***25.6 ± 9.59***	***42.12 ± 9.26***	**F= 8.57 ; p= 0.004**	F= 0.02 ; p= 0.883

ANALYZING DIFFERENCE MILKING - WAITING

Considering the difference between milking phases and waiting periods, the mean HR increased significantly more in NP cows than in control animals (p=0.007, Table 17). Statistical analysis did not reveal further differences. Close to statistical tendency, the LF/HR ratio decreased in nose pressing animals while it increased in the control group.

When taking bouts of NP cows with continuous nose pressing and without nose pressing taken into account (Table 18), both STD HR and RMSSD decreased with nose pressing and slightly increased as compared to the waiting period when the nose pressing behaviour was shown and was completely absent, respectively. An almost similar pattern was observed for STD RR (p=0.055).

Finally, in Table 19 bouts with nose pressing of at least 15s duration (labels 1 and 2) are compared to those bouts where nose pressing duration was shorter than fifteen seconds or without nose pressing behaviour observed (labels 3 and 4). In this case, the only statistical difference was found for RMSSD, which decreased as compared to the waiting period when nose pressing was shown for at least 50% of the bout (labels 1 and 2) and increased with a lower proportion of nose pressing or no nose pressing (labels 3 and 4).

Table 17. Change of HRV values from waiting to milking for NP and control cows and effects of "group and "period" (morning, afternoon)

	Group			Period			
HRV variable	**NP**	**Control**	**Effect of "Group"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	***1.62 ± 0.23***	***0.58 ± 0.27***	**F= 8.50; p= 0.007**	*0.91 ± 0.24*	*1.29 ± 0.27*	F= 1.15; p= 0.292	F= 0.00; p= 0.962
STD HR (1/min)	*-0.11 ± 0.05*	*-0.11 ± 0.05*	F= 0.60; p= 0.440	*-0.06 ± 0.05*	*-0.10 ± 0.75*	F= 0.24; p= 0.624	F= 1.01; p= 0.315
STD RR (ms)	*-0.80* ± *0.32*	-0.29 ± *0.32*	F= 1.35; p= 0.248	-0.50 ± *0.34*	-0.60 ± *0.29*	F= 0.05; p= 0.831	F= 0.46; p= 0.498
RMSSD (ms)	*-0.34 ± 0.18*	*-0.12 ± 0.16*	F= 0.83; p= 0.367	*-0.17 ± 0.20*	*-0.29 ± 0.13*	F= 0.23; p= 0.629	F= 0.10; p= 0.759
HF (%)	*0.28* ± 0.41	*0.04 ± 0.34*	F= 0.20; p= 0.659	***1.22*** **± 0.49**	**-0.90 ± 0.23**	**F= 15.6; p= 0.000**	F= 0.03; p= 0.865
LF (%)	*0.91 ± 0.61*	*2.01 ± 0.57*	F= 1.76; p= 0.187	*1.88 ± 0.63*	*1.04 ± 0.55*	F= 1.02; p= 0.313	F= 0.28; p= 0.599
LF/HF ratio	*-3.35 ± 2.21*	*1.69* ± 2.06	F= 2.77; p= 0.102	*-1.22 ± 2.26*	*-0.45 ± 2.01*	F= 0.06; p= 0.802	F= 4.48; p= 0.309

Table 18. Change of HRV values in NP cows from waiting to milking and effects of nose pressing and period (morning, afternoon) when bouts with continuous and no nose pressing were taken into account only (1: complete nose pressing bout; 4: no nose pressing observed)

	Label			Period			
HRV variable	**1**	**4**	**Effect of "Label"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	*1.23 ± 0.73*	*1.98* ± *0.61*	F= 0.62; p= 0.434	*2.39 ± 0.76*	*0.82 ± 0.58*	F= 2.708;1= 0.104	F= 0.11; p= 0.740
STD HR (1/min)	***-0.21 ± 0.10***	***0.07 ± 0.09***	**F= 4.36; p= 0.040**	*-0.07 ± 0.11*	*-0.08 ± 0.08*	F= 0.00; p= 0.951	F= 0.00; p= 0.967
STD RR (ms)	*-1.77 ± 0.65*	*-0.12* ± 0.65	F= 3.79; p= 0.055	*-1.19 ± 0.68*	*-0.69 ± 0.51*	F= 0.35; p= 0.557	F= 0.05; p= 0.831
RMSSD (ms)	***-0.55 ± 1.15***	***1.04 ± 1.13***	**F= 7.86; p= 0.006**	*0.38 ± 1.17*	*0.11 ± 1.11*	F= 0.21; p= 0.650	F= 0.57; p= 0.453
LF (%)	*1.35 ± 0.18*	*0.77 ± 1.29*	F= 0.44; p= 0.508	*2.37 ± 1.60*	*0.50 ± 1.22*	F= 0.87; p= 0.355	F= 0.38; p= 0.541
HF (%)	*1.76 ± 1.88*	*1.20 ± 1.82*	F= 0.18; p= 0.673	*1.74* ± 1.99	*1.22 ± 1.77*	F= 0.15; p= 0.703	F= 1.72; p= 0.194
LF/HF ratio	*-3.91 ± 6.25*	*4.24* ± 5.26	F= 1.00; p= 0.321	*3.82 ± 6.50*	*-3.49 ± 4.95*	F= 70.8; p= 0.373	F= 0.03; p= 0.862

Table 19. Change of HRV values in NP cows from waiting to milking and effects of nose pressing and period (morning, afternoon) when bouts with continuous, discontinuous and no nose pressing were taken into account (1: complete nose pressing bout; 2: nose pressing observed >50% of the bout; 3: nose pressing observed <50% of the bout; 4: no nose pressing observed)

	Label			Period			
HRV variable	**1-2**	**3-4**	**Effect of "Label"**	**Morning**	**Afternoon**	**Effect of "Period"**	**Interaction effect**
Mean HR (1/min)	*1.31 ± 0.64*	*1.94 ± 0.58*	F= 0.52; p= 0.473	*2.34 ± 0.69*	*0.91 ± 0.52*	F= 2.75; p= 0.100	F= 0.09; p= 0.761
STD HR (1/min)	*-0.16 ± 0.07*	*-0.01 ± 0.06*	F= 2.97; p= 0.088	*-0.08 ± 0.07*	*0.08 ± 0.05*	F= 0.00; p= 0.997	F= 0.02; p= 0.886
STD RR (ms)	*-1.54 ± 0.40*	*-0.66 ± 0.40*	F= 2.19; p= 0.142	*-1.47 ± 0.47*	*-0.72 ± 0.36*	F= 1.61; p= 0.207	F= 0.09; p= 0.765
RMSSD (ms)	***-1.36 ± 0.34***	***0.40 ± 0.31***	**F= 4.40; p= 0.039**	*-1.00 ± 0.37*	*-0.76* ± 0.28	F= 0.28; p= 0.595	F= 0.02; p= 0.967
LF (%)	*1.50 ± 1.19*	*-0.36 ± 1.10*	F= 1.32; p= 0.254	*1.06 ± 1.29*	*0.08 ± 0.98*	F= 0.37; p= 0.546	F= 0.02; p= 0.877
HF (%)	*-0.68 ± 0.85*	*0.65 ± 0.78*	F= 1.33; p= 0.251	*0.17* ± 0.92	*0.21* ± 0.70	F= 0.11; p= 0.743	F=0.06; p= 0.808
LF/HF ratio	*0.16 ± 4.83*	*2.11 ± 4.44*	F= 0.09 p= 0.767	*4.92 ± 5.23*	*-2.65 ± 3.96*	F= 1.33; p= 0.251	F= 0.05; p= 0.827

ANALYZING LYING

Data related to lying HRV parameters has been analyzed using a Student's t-test and gathered in

Table 21. There was no significant difference between NP and control cows in the parameters both during the day and during the night.

Table 20. HRV variables during lying. Analysis during periods (day, night)

	Day		Night	
HRV variable	**NP**	**Control**	**NP**	**Control**
Mean HR (1/min)	79.2 ± 3.56	82.3 ± 4.27	79.2 ± 3.87	81.7 ± 3.90
STD HR (1/min)	2.47 ± 0.68	2.47 ± 0.45	2.16 ± 0.64	2.6 ± 0.97
STD RR (ms)	13.2 ± 4.57	14.2 ± 1.98	13.1 ± 3.89	16.4 ± 6.16
RMSSD (ms)	6.73 ± 2.17	7.24 ± 1.66	7.08 ± 4.62	9.68 ± 5.58
LF (%)	66.0 ± 6.34	67.1 ± 3.82	66.2 ± 4.53	66.3 ± 5.69
HF (%)	4.90 ± 2.48	4.39 ± 2.07	5.43 ± 2.55	5.59 ± 1.83
LF/HF ratio	28.0 ± 15.0	25.4 ± 11.7	24.0 ± 9.66	23.7 ± 8.37

Table 21. HRV parameters during lying. Student's t-test

	HRV variable	t-Value	p
Day	**Mean HR (1/min)**	-1.439	0.176
	STD HR (1/min)	-0.009	0.993
	STD RR (ms)	-0.549	0.593
	RMSSD (ms)	-0.486	0.635
	LF (%)	-0.407	0.691
	HF (%)	0.421	0.681
	LF/HF ratio	0.375	0.714
Night	**Mean HR (1/min)**	-1.125	0.283
	STD HR (1/min)	-1.124	0.283
	STD RR (ms)	-1.367	0.197
	RMSSD (ms)	-1.219	0.246
	LF (%)	-0.037	0.971
	HF (%)	-0.126	0.902
	LF/HF ratio	0.053	0.958

BEHAVIOURS SHOWN IN THE BARN ENVIRONMENT

NOSE PRESSING

Four NP cows (Cows 1, 11, 13 and 27) showed nose pressing behaviour in the barn (Table 22). There are considerable differences in the number of events observed and in the total duration of nose pressing. Some of the bouts were shown while the cow was lying and some of them were observed while the animal was standing; in addition, all the events were performed against the walls or the metal rods of the cubicles or the feeding rack. One cow, which had not been selected as focal animal, showed the behaviour onto the back of another animal (Fig. 10).

Table 22. The number and total time observed of nose pressing bouts, frustrating events and tongue rolling during the observation period (10 days) in the groups of cows observed.

		Total Time Observed (h)	Nose Pressing bouts		Frustrating events		Tongue rolling
Group	Cow		Times observed	Total time (min)	Feeding Machine	Cubicle	Times observed
NP	1	13.83	2	1			2
	11	15.92	7	21.33	3		
	13	11.25	4	6.5			1
	19	14.17					
	23	11.83					
	27	14.33	5	8.67			
	69	14.42					1
Control	7	15.25				1	
	29	12.42			1		
	34	14.17					1
	35	12.58					
	41	14.25					
	64	14.50					2
	84	11.75					

POTENTIALLY FRUSTRATING EVENTS

During the observation period, cow 11 showed aggressive behaviours against the feeding machine three times; cow 29 behave similarly but only once. On the other hand, cow 7 was observed showing difficulties to rise from the cubicle.

Fig. 10. Brown Suiss dairy cow performing nose pressing onto the bone structure of the tail base

TONGUE ROLLING

Cows 1, 13 and 69 from the nose pressing group and cows 34 and 64 from the control group were seen performing tongue rolling during the observation period. As seen in Table 22, cows 1 and 64 performed the behaviour two times and the rest of the animals were seen only once.

SOCIAL INTERACTIONS

a) Displacement

All the animals of both groups where seen performing and suffering displacements during the observation period. These behaviours were observed all over the barn, although the number increased in the sectors with feeding rack.

Table 23 and Table 24 show the social ranking of the cows calculated by Eq. 1 and Eq. 2. In both cases, the ranking obtained is similar. Both for the Galindo-Broom and Mendl approach, the range of 'active' events exerted by nose pressing cows is larger than the range of the control cows. However, regarding passive actions the range is similar in both indices. There are no considerable differences between the ranking of the nose pressing and the control group. In both groups no clear pattern explaining a relation between age and social ranking was found.

Table 23. Number of times a cow displaces any other cow and number of times the individual has been displaced as well as social ranking based on Galindo-Broom's index for NP and control animals.

Group	Cow	Breed	Date of birth	Active[4]	Passive[5]	Galindo-Broom	Ranking
NP	1	AF	25/05/2008	4	7	0.36	LOW
	11	AF	13/12/2008	32	5	0.87	HIGH
	13	AF	16/08/2010	5	3	0.63	HIGH
	19	BS	28/02/2004	12	2	0.86	HIGH
	23	AF	04/11/2010	5	1	0.83	HIGH
	27	AF	13/11/2010	12	15	0.44	MIDDLE
	69	AF	14/01/2007	9	3	0.75	HIGH
Control	84	AF	10/05/2008	17	9	0.65	HIGH
	35	AF	15/06/2005	10	2	0.83	HIGH
	7	AF	26/07/2009	5	9	0.36	LOW
	34	BS	10/05/2011	8	15	0.35	LOW
	41	AF	27/09/2005	12	5	0.71	HIGH
	64	AF	26/07/2009	13	3	0.81	HIGH
	29	AF	22/02/2005	13	3	0.81	HIGH

Table 24. Number of times a cow displaces any other cow and number of times the individual has been displaced as well as social ranking based on Mendl's index for NP and control animals.

Group	Cow	Breed	Date of birth	Active[6]	Passive[7]	Mendl	Ranking
NP	1	AF	25/05/2008	3	7	30.0	LOW
	11	AF	13/12/2008	24	5	82.8	HIGH
	13	AF	16/08/2010	5	3	62.5	HIGH
	19	BS	28/02/2004	10	2	83.3	HIGH
	23	AF	04/11/2010	4	1	80.0	HIGH
	27	AF	13/11/2010	9	12	42.9	MIDDLE
	69	AF	14/01/2007	7	3	70.0	HIGH
Control	84	AF	10/05/2008	11	9	55.0	MIDDLE
	35	AF	15/06/2005	9	3	75.0	HIGH
	7	AF	26/07/2009	5	5	50.0	MIDDLE
	34	BS	10/05/2011	7	11	38.9	LOW
	41	AF	27/09/2005	9	4	69.2	HIGH
	64	AF	26/07/2009	11	4	73.3	HIGH
	29	AF	22/02/2005	13	3	81.3	HIGH

[4] Number of times the individual displaces any cow
[5] Number of times the individual has been displaced
[6] Number of cows that an individual is able to displace
[7] Number of cows that are able to displace the individual

b) Allogrooming

With the exception of one cow (cow 13), who only received social licking, all animals were seen performing and receiving allogrooming. Within the data set obtained (Table 25 and Table 26), it is observed that the variation of the number of events observed is wider in the nose pressing group than in the control group. At the same time, the range of the number of cows that an individual is able to lick is broader in cows that showed nose pressing behaviour than in cows selected as control animals. However, the passive events observed in each group are similar in Index 1 and Index 2. The values of the indices obtained through the Eq. 3 and Eq. 4 has been ranked but it has not been found any criterion to correlate both assortments.

Table 25. Number of times a cow grooms any other cow and number of times the individual has been groomed as well as allogrooming ranking based on the proportion of acting licking events for NP and control animals.

Group	Cow	Breed	Date of birth	Active[8]	Passive[9]	Index 1	Ranking
NP	1	AF	25/05/2008	14	5	0.74	HIGH
	11	AF	13/12/2008	5	7	0.42	MIDDLE
	13	AF	16/08/2010	0	7	0.00	LOW
	19	BS	28/02/2004	26	13	0.67	HIGH
	23	AF	04/11/2010	3	5	0.38	LOW
	27	AF	13/11/2010	3	2	0.60	MIDDLE
	69	AF	14/01/2007	10	7	0.59	MIDDLE
Control	84	AF	10/05/2008	4	2	0.67	HIGH
	35	AF	15/06/2005	4	6	0.40	MIDDLE
	7	AF	26/07/2009	3	2	0.60	MIDDLE
	34	BS	10/05/2011	8	4	0.67	HIGH
	41	AF	27/09/2005	1	1	0.50	MIDDLE
	64	AF	26/07/2009	2	4	0.33	LOW
	29	AF	22/02/2005	4	7	0.36	LOW

[8] Number of times the individual likes any cow
[9] Number of times the individual has been licked

Table 26. Number of times a cow grooms any other cow and number of times the individual has been groomed as well as allogrooming ranking based agonistic interactions for NP and control animals.

Group	Cow	Breed	Date of birth	Active[10]	Passive[11]	Index 2	Ranking
NP	1	AF	25/05/2008	9	5	64.3	HIGH
	11	AF	13/12/2008	4	5	44.4	MIDDLE
	13	AF	16/08/2010	0	6	0.0	LOW
	19	BS	28/02/2004	17	8	68.0	HIGH
	23	AF	04/11/2010	3	4	42.9	MIDDLE
	27	AF	13/11/2010	2	2	50.0	MIDDLE
	69	AF	14/01/2007	9	7	56.3	MIDDLE
Control	84	AF	10/05/2008	3	2	60.0	MIDDLE
	35	AF	15/06/2005	3	6	33.3	LOW
	7	AF	26/07/2009	3	2	60.0	MIDDLE
	34	BS	10/05/2011	5	4	55.6	MIDDLE
	41	AF	27/09/2005	1	1	50.0	MIDDLE
	64	AF	26/07/2009	2	4	33.3	LOW
	29	AF	22/02/2005	4	6	40.0	MIDDLE

Table 27. Number of head-play events observed in the barn during the observation period (10 days) and cows involved in the actions.

		Cow B													
Group	**Cow A**	**6**	**16**	**22**	**40**	**47**	**50**	**58**	**70**	**78**	**81**	**90**	**93**	**94**	**Total**
NP	**1**	1												1	2
	11			1						2					3
	13														
	19											2			2
	23				1				1			1			3
	27						1			1					2
	69												1		1
Control	**7**														
	29		1			1		1							3
	34														
	35										1	1			2
	41														
	64														
	84														

[10] Number of cows that an individual is able to lick

[11] Number of cows that are able to lick the individual

a) Head-play

Almost every cow of the nose pressing group, excluding cow 13, was seen performing head-play during the observation period; on the contrary, from the control group only the cows 29 and 35 performed this behaviour. The great majority of the actions were observed after the afternoon milking period.

b) Mounting activity

Only few events of mounting activity were observed. All the events were performed or received by nose pressing cows 11 and 13 as seen in Table 28 and

Table 29.

Table 28. Number of mounting events performed by nose pressing cows

		Actor				
	Receiver	**22**	**76**	**78**	**88**	**Total**
NP	**1**					
	11	5		1	2	8
	13	2	2			4
	19					
	23					
	27					
	69					

Table 29. Number of mounting events received by nose pressing cows

		NP (Actor)						
		1	**11**	**13**	**19**	**23**	**27**	**69**
Receiver	**22**		2					
	76			1				
	81			1				
	83		1					
	Total		3	2				

Sector Observation

The results collected in Table 30 have been subjected to a Chi-Square test in order to verify whether there is a relation between the animals in each group and the distribution of the percentages obtained. Both nose pressing and control groups follow the same distribution (p=0.729, Table 31 and Table 32).

Table 30. Collection of focused dairy cows and the number of animals labelled depending on the percentage of times observed together.

Group	Cow	0-5%	5-20%	20-40%	40-60%
NP	1	11	29	31	0
	11	14	21	34	2
	13	14	29	27	1
	19	12	30	27	2
	23	13	30	24	4
	27	15	32	23	1
	69	14	27	30	0
Control	7	14	30	27	0
	29	19	25	25	2
	34	13	20	34	4
	35	13	24	33	1
	41	17	21	32	1
	64	16	32	23	0
	84	10	30	30	1

Table 31. Cross tabulation group (nose pressing, control) and labelling (L0, L1, L2, L3)

			Level				Total
			L0	L1	L2	L3	
Group	Control	Count	102	182	204	9	497
		Expected Count	97,5	190,0	200,0	9,5	497,0
	NP	Count	93	198	196	10	497
		Expected Count	97,5	190,0	200,0	9,5	497,0
Total		Count	195	380	400	19	994
		Expected Count	195,0	380,0	400,0	19,0	994,0

Table 32. Chi-Square Test (group and labelling)

	F	df	p
Pearson Chi-Square	1.302	3	0.729
Likelihood Ratio	1.302	3	0.729
N of Valid Cases	994		

LYING AND STANDING PHASES

The mean values of the duration and bouts observed during the resting time are similar for both groups. An account of neither of the p-values are lower than the statistical significance (α=0.05) it can be affirmed that there is no difference between both groups (nose pressing and control) in the time that the animals spend lying on their cubicles (mean lying), in the number of lying bouts observed or in the total time the animals spend lying.

Nevertheless, whether each group is deeper analysed, some differences are able to be discerned. In this way, regarding the nose pressing collection, cows 11 and 19 showed a considerable reduction of the total lying time when facing with cow 1. Similarly, in the control group, cows 29, 34, 35 and 64 spent resting less hours than cow 41. All in all, the variability of the nose pressing group is high, however, the variability of the control animals is higher.

Table 33. Collection of means (lying, number of bouts and total duration) per animal gathered in nose pressing or control group.

Group	Cow	Mean bout length (h)	Mean number of bouts	Mean Total lying time (h)
NP	1	1.49	8.10	12.1
	11	1.43	6.53	8.73
	13	1.13	9.42	10.1
	19	1.40	7.05	9.36
	23	1.14	10.6	11.5
	27	0.99	11.1	10.5
	69	1.26	9.05	10.8
Control	7	1.04	10.6	11.0
	29	1.40	6.30	8.43
	34	1.32	7.26	9.09
	35	1.69	5.95	9.52
	41	1.33	10.2	12.9
	64	0.92	8.90	7.82
	84	1.63	7.20	11.7

Table 34. Student's t-Test; df =3 (mean lying time, mean bouts and mean total duration of lying per day)

	F	p
Mean Lying	-0.565	0.583
Mean Bouts	0.827	0.424
Mean Total Duration	0.447	0.663

DISCUSSION

This part of the thesis shows a deeply discussion of the results obtained in previous chapters and it diverts on concrete conclusions about nose pressing behaviour and its relation with HRV, milking, resting and different behaviours in cattle.

HRV ANALYSIS

To begin with, through the analysis of the results obtained it is easy to observe an increase of the sympathetic activity, as much in nose pressing animals as in control group, during the waiting times before afternoon milking as compared to the waiting period before morning milking. During the afternoon milking, dairy cows showed a significant increment of HR and LF/HF ratio, as well as a decrease in RMSSD when making a comparison with morning waiting period. In the same way, the effect is appreciably similar when cows were being milked; again, the afternoon values of heart rate were higher and RMSSD values were lower as compared to morning values. There is a clear tendency of a higher sympathetic activity in the afternoon periods that was suspected at the beginning of the research. In a similar way, this hypothesis was previously proved (Gutmann et al. 2013), in spite of the tendency was strong in milking procedures but not so clear during waiting periods.

These previous results corroborate what Kovács, Kézér & Tőzsér (2013) proved. Parasympathetic activity decreases concurrently the day draws on and it could be related with the increasing of the amount of actions that an animal carries out as well. Although in this research the variation of social actions (displacements, head play, allogrooming…) throughout the day has not been taken into account, during the observation period it was clearly observed. Nevertheless, the increase of sympathetic activity in the afternoon period does not equate to a higher stress level. The increasing of the social events observed in the barn environment is not enough to claim that dairy cows reacted in a different way against the stressors depending on the day period.

Apart from morning-afternoon variation, during waiting periods control animals showed a higher mean HR than nose pressing cows. The difference is considerable; control animals presented five beats per minute more than nose pressing animals, and it is translated again into a higher sympathetic activity in control cows. A key point to explain this result might be a deep analysis of the preceding activity to the milking time. It should be taken into account not only the previous time to the entrance into the milking parlour, but also the behaviour during waiting.

Separately to these possible investigations, and in order to give the results a global point of view of the waiting periods, it is needed to consider the other parameters related to HR: STD HR during waiting was – close to statistical tendency level ($p=0.102$) – slightly lower in nose pressing animals, whereas Gutmann, et al., 2013, reported a higher parasympathetic level observed in control animals and a tendency of a higher STD RR in the same group.

The analysis of HRV parameters during milking time yielded similar results as compared to the waiting periods. On the first hand, control group showed a higher mean HR and the difference between both collections were again close to five beats per minute. For no other HRV variables statistically significant differences were found, however, the analysis of the difference between milking and waiting times drops some valuable information. Although nose presser's heart rate was lower than the control animals, the magnitude of the difference was higher in the nose pressing group than in the control group. In other words, milking procedures had a greater impact on nose pressing animals than on other cows. This was previously predicted (Gutmann et al. 2013) who reported the same tendency in the difference of heart rate between milking and waiting.

On the whole, it seems that nose pressing cows differ from other animals through the lower mean heart rate and the higher increase of heart rate during milking periods. In the same way, the results obtained contain differences with previous studies (Gutmann et al. 2013); however, these variations might appear on account of differences in the base line of both researches. Therefore, it might explain why the interaction effects did only occur in Gutmann et al. (2013). In this manner, cows identified as exhibiting nose pressing might depart from a balanced level (non-stressed) during the waiting time. In this case, the milking procedure might rouse the nose pressing group more than other cows becoming in higher heart rate values.

With reference to the nose pressing action during milking, it was observed that pure nose pressing bouts entailed lower values of RMSSD, STD HR and STD RR compared to those periods where the behavior was not shown. This lower value of RMSSD is related to a higher sympathetic activity and, in the same direction, with a higher percentage of the low frequency spectrum which is finally transformed in a higher heart rate. The lower STD HR and RR intervals observed indicate a more balanced activity during the performance of nose pressing behavior. These results were less pronounced when bout-categories 1 and 2 were compared with categories 3 and 4 (Table 4); as a matter of fact, none of the HRV variables differed at a statistically significant level. However, RMSSD and STD HR showed the same previous tendency, which gives some useful information: essentially, the results of the analysis of pure bouts (categories 1 and 4) yielded higher differences between STD HR and RMSSD mean values for nose pressing and non-nose pressing bouts at a higher significance level than the analysis of mixed bouts, indicating that the observed effect was directly caused by nose pressing.

In this way, to compare the results with those obtained by Gutmann, et al., 2013, may be controversial because they found higher values of parasympathetic activity during the nose pressing performance. Again, the previously explained baseline-interaction effect might have an influence on the results. In this way, stressed-dairy cows might show nose pressing and continue showing over time independently of their stress level. Anyway, although results seem to be different, it might be confirmed that nose pressing has an effect on animal's physiology.

Finally, the analysis of HRV during resting time did not show any new information to support the statemets previously exposed. Separate evaluation of HRV parameters during lying day and night periods seem to follow the same statistical distribution for NP and control animals; thus, it cannot be claimed that there are differences in the sympathetical or parasympathetical activity in both periods. However, the mean HR observed during resting is slightly greater than the value obtained during waiting or milking procedures.

In addition to HRV examination during resting, the analysis of the lying periods do not provide consistent statements. To a certain extent, the variability of the data set obtained regarding the nose pressing animals might offer some helpful information.

BEHAVIOUR ANALYSIS IN THE BARN ENVIRONMENT

To begin with, nose pressing was observed several times in the barn environment; consequently, it can be assumed that nose pressing behaviour is not due to the milking procedure as itself. In other words, the performance of nose pressing is the result of some incomes that affect the animals in different ways.

Potential frustrating events were detected during the observing time in the barn; the amount of events identified is not enough to ensure that they are unequivocal nose presser factors though. As far as it is concerned, these events are hold as stressor factors that have an influence on animals and, in this way, dairy cows react against the situation.

In a similar way, tongue rolling performance is understood as a stereotypical behaviour which is apparently uncoupled from biological functions. Nevertheless, the repetitive observations made in the barn may help to elucidate that this behaviour is the response carried out in order to cope with, possibly, frustration. However, it is not possible to purely relate nose pressing behaviour with tongue rolling because both group showed it.

In order to obtain a global view of the barn environment, another point is that social hierarchy rules the way dairy cows socialize. The social ranking calculations did not offer any obvious relations to nose pressing action. On the other hand, to observe the values of the active displacements carried out may be used for deducing new facts. In this case, it is surprising that nose pressing cow 11 showed almost three times more active displacements than any other cow in its same group. Naturally, it has no statistical valuable information to incorporate but it is, at least, something to be taken into account. To a certain extent, it might be interesting to know the preferences that animals of both groups had to be in contact with other dairy cows. In this way, through the sector analysis made, it is proved that as much nose pressers as control animals would rather be connected with some cows and try to avoid others.

Again, allogrooming evaluation did not offer consistent information to be related with nose pressing. Both statistical distributions seem to be similar but, otherwise, the range that the nose pressing values encompass (active and passive) is wider than the values obtained by the observation of the control group. It might be interesting to emphasize cow 19 because of its active values are double than any other.

Finally, head play events were performed in their majority by nose pressing cows and mounting activity was observed only on nose pressers. The estrus phase might be an explanation of the higher activity shown by some of the nose pressing animals, but the reproductive calendar were checked and no cow were supposed to be on its sexual peak.

Fig. 11. Austrian Fleckvieh and Brown Suiss performing head play behaviour

On the whole, the behaviour analysis in the barn environment does not offer a clear view of whether nose pressing cows are under the influence of a higher amount of stressors or, otherwise, if they react in a different way. On the other hand, the great variability shown within the nose pressing group compared with other animals might suppose that nose pressing behaviour is the reaction that dairy cows carry out in response to stressors; nonetheless, the stress level that each dairy cow tolerates might differ. So that, within the nose pressing individuals, the stress level to which dairy cows are subject to might vary.

BEHAVIOUR ANALYSIS IN THE MILKING PARLOUR

The behaviours observed in the milking parlour seem to be a bit controversial; actually, the statistical analysis is not able to offer differences between nose pressing and control animals. The behaviours observed inside each group (nose pressing, control) differ from one individual on another; in other words, the variability of the values obtained prevents to examine the behaviours in a group-way.

For instance, Fig. 12 represents the relation between the number of nose pressing bouts observed in each milking and the sum of all the movement behaviours (stepping and /or kicking) detected in the same milking. These values are divided into two series, the first one collects data from those milking phases where the hander supplied feed to the animal; the second is related with those ones where feed was not provided. As it is observed, it might be perceived a tendency which relates the increase of nose pressing bouts with an increase of kicking or stepping events. This relation might connect the performance of nose pressing with a higher stress level which would be revealed by the increase of stepping and kicking behaviours. However, there are some values that do not allow affirming the statement with accuracy.

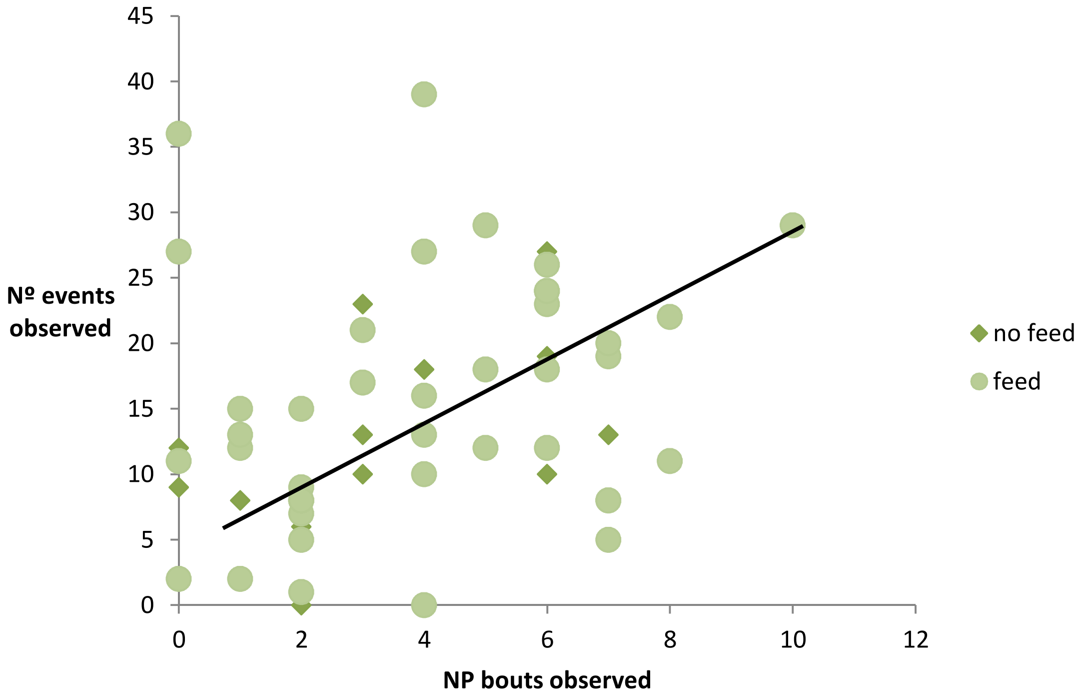

Fig. 12. Graphic based on the number of nose pressing bouts observed per milking and it relation with the number of events observed (stepping and/or kicking) conditional to the feed provision.

NOSE PRESSING

In brief, all the previous chapters contribute to get a global idea of the effect of nose pressing and the events that may elicit, stimulate or affect the performance of this behavior. Although the research may be focused on different dimensions of the dairy cows' day-to-day life, it is needed to gather all these strategic knowledge in order to obtain a global idea about the reasons why this behavior appears. In the same way, the final reactions that nose pressing triggers on the animal's physiology are required to be explained.

In this research, it has been found that the performance of nose pressing behavior results in the reduction of STD HR, STD RR and RMSSD which means a lower parasympathetic activity. In the same manner, nose pressing dairy cows apparently are under a similar influence of stressors than other cows that did not show the behavior. In addition, it seems that nose pressing cows reacts in a comparable way (excluding nose pressing performance) to the animals in the control group; e.g. similar distribution of the social hierarchy with in the groups, similar oral behaviors etc.

However, the fact that some cows, which were gathered at the beginning of the research as nose pressing or control, had to be excluded from the study because they changed their behaviour must be taking into account. Similarly, some animals were observed only showing the behaviour in the barn but no during milking procedures. In addition, looking at the cows analyzed by Gutmann, et al. (2013), and contrasting them with those animals used in this study, it has been found that cow 27 were, in the first case, classified as control and, in the second, as a nose presser. For these reasons, the option that nose pressing is a transitory behaviour should not be disregarded; in fact, it might explain the similarities in the behavioural observations made (NP and control).

Concerning the results obtained from anaylsis of HRV parameters and the differences in results as compared to the study of Gutmann et al. (2013), an option might be that the performance of the behaviour did not have the same effect on all the dairy cows. In this way, some dairy cows might performe nose pressing behavior independently of the stress level they beared. The variability of the results obtained might ratify this statement, however, it should be needed to enlarge the sample size and follow again the protocol to ensure this possibility.

CONCLUSIONS

Here the answer to the questions posed at the beginning of the study can be found. The detailed discussion of the objectives has been developed in the previous chapter, so that, in order to understand completely this section, the previous chapter must be read first.

CONCLUSIONS

This study investigated the reasons and the effect of nose pressing behavior in terms of heart rate variability and behavior. To sum up, nose pressing animals showed different HRV values during resting, waiting and milking times and these magnitudes were modified by the effect of the nose pressing action. On the other hand, it has not been proved that relaxing systemic changes were triggered trough the performance of nose pressing, however, it can be claimed that it had an effect on animal's physiology. The only difference found in the response that animals of the same herd carried out in order to react against the same stimuli was the performance of nose pressing behaviour.

In addition, it was founded that nose pressing is not only performed in the milking parlour; nevertheless, the number of nose pressing bouts observed in the barn was minor.

There was not found any connection between social hierarchy in the herd with the performance of the behavior. Similarly, the performing of allogrooming between animals of the same herd is not related as well. Likewise, the frequency and duration of the resting phases did not differ between nose pressing cows and other animals of the same herd.

Lastly, it is found that nose pressing behavior is a transitory behavior which not always unchain the same reactions on dairy cows.

APPENDIX A

In this section results from (Gutmann et al. 2013) are detailed. That includes means of HRV parameters for main effects and significant interaction effects for waiting, milking, and the difference between milking and waiting. Additionally, means of HRV parameters for main effects and significant interaction effects for nose-pressing animals during milking when showing nose-pressing or not are incorporated.

In addition, it includes the sort of animals observed and the collections made.

GUTMANN, ET AL. (2013) RESULTS.

Table 35. Means of HRV parameters for main effects and significant interaction effects for waiting, milking, and the difference between milking and waiting. C = control animals, NP = nose-pressing animals, CT = cow type (NP/C), M = morning, A = evening. Significant effects are highlighted in bold for p<0.05, and italic for p<0.1 (Gutmann et al. 2013).

HRV parameters		Waiting			Milking			Difference between milking and waiting		
	Factor	NP	C	Period	NP	C	Period	NP	C	Period
HR	CT	75.1	74.9		79.7	77.0		*2.1*	*0.6*	
	M			**72.3**			**75.6**			1.6
	A			**78.4**			**81.1**			1.1
RMSSD	CT	7.3	9.8		7.3	8.4		-0.5	-0.4	
	M	**7.3**	**11.1**	9.2			**8.7**			-0.7
	A	**7.3**	**8.5**	7.9			**7.1**			-0.2
STD HR	CT	2.7	2.5		2.0	2.3		-0.7	-0.8	
	M			2.6			**2.0**			-0.9
	A			2.6			**2.3**			-0.6
STD RR	CT	*14.9*	*20.4*		**13.7**	**18.9**		-0.2	-0.2	
	M	*14.8*	*21.2*	18.0			16.3			-0.1
	A	*15.1*	*19.5*	17.3			16.3			-0.2
HF%	CT	6.6	5.1		6.4	4.9		-0.6	-0.1	
	M			6.3	**7.6**	**5.1**	**6.3**	**1.0**	**-1.2**	-0.1
	A			5.4	**5.3**	**4.8**	**5.1**	**-2.1**	**1.0**	-0.6
LF%	CT	69.7	66.1		71.0	68.7		3.2	2.9	
	M			**66.8**			68.0	*1.3*	*5.2*	4.0
	A			**68.9**			71.7	*3.5*	*1.9*	2.2
LF/HF	CT	28.8	22.5		**18.9**	**33.0**		-1.6	0.1	
	M			**20.1**			**20.9**	**-1.3**	**5.1**	2.3
	A			**31.2**			**30.7**	**0.1**	**-5.3**	-3.7

Table 36. Means of HRV parameters for main effects and significant interaction effects for nose-pressing animals during milking when showing nose-pressing (NP+) or not (NP-). CT = cow type (NP/C), M = morning, A = evening. Significant effects are highlighted in bold for p<0.05, and italic for p<0.1 (Gutmann et al. 2013).

Time based parameters					Frequency based parameters				
HRV parameters		Milking			HRV parameters		Milking		
	Factor	NP+	NP-	Period		Factor	NP+	NP-	Period
HR	CT	**77.7**	**80.1**		HF%	CT	**6.9**	**5.2**	
	M			**75.4**		M			**7.3**
	A			**82.3**		A			**4.7**
RMSSD	CT	*7.3*	*6.5*		LF%	CT	*64.5*	*69.5*	
	M	**8.5**	**6.7**	**7.58**		M	*59*	*68.7*	**63.8**
	A	**6.2**	**6.3**	**6.25**		A	*70.1*	*70.3*	**70.2**
STD HR	CT	**1.8**	**2.1**		LF/HF	CT	14.8	18.3	
	M			**1.7**		M			**12.9**
	A			**2.1**		A			**20.2**
STD RR	CT	12.4	14						
	M			12.4					
	A			14					

Table 37. Collection of animals selected and the groups gathered. (Gutmann et al. 2013)

Group	Cow
NP	11
	23
	91
	92
	79
	95
	13
	55
	69
Control	5
	86
	81
	48
	65
	27
	47
	77
	84
	94

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